WHITEPAPER

Troubleshooting: Fixation

BIOCARE MEDICAL

Troubleshooting: Fixation

As the first preparatory step in the immunohistochemistry (IHC) process, proper tissue fixation is critical. Fixation ensures that the tissue's cellular structure and composition are preserved for visualization. The ideal fixative will harden tissue to preserve these structures while also preventing tissue degradation.[2] Any errors in the fixation process will affect the staining quality of all other steps downstream.

The most severe staining issues are the result of delayed or incomplete fixation. When tissue is appropriately fixed, multiple destructive processes should be prevented by the fixative solution, including putrefaction and autolysis. Putrefaction occurs when bacteria or fungi consume the tissue. Autolysis is the self-digestion or self-destruction of a cell through the action of its own enzymes. Naturally occurring enzymes present in the tissue will continue their metabolic processes even after the tissue is removed, breaking down tissue components until they are inhibited by the fixative.[1] If fixation is delayed, and either of these processes is allowed time to occur, cell structures may be irrevocably lost. Bacteria or fungi may be visible in the stain if they are present. If the tissue has autolyzed, some cells may disappear, and tissue components may appear to shrink, creating artificial space around the cells.[1]

Even after the tissue is placed in the fixative solution in a timely manner, it must be given ample time for both the fixative to fully penetrate the tissue and for the fixative action to take place. For formalin fixation, penetration occurs at an approximate rate of 1mm per hour. It may take up to 24 to 48 hours for initial crosslinking to be completed after full penetration is achieved.[1,2] If the specimen is too large, with insufficient surface area relative to volume, it will impede penetration, and fixation may be delayed or incomplete. Tissue showing signs of incomplete fixation will appear to have poor morphology. Nuclei may appear smudgy or seem to "bubble" when stained.[1]

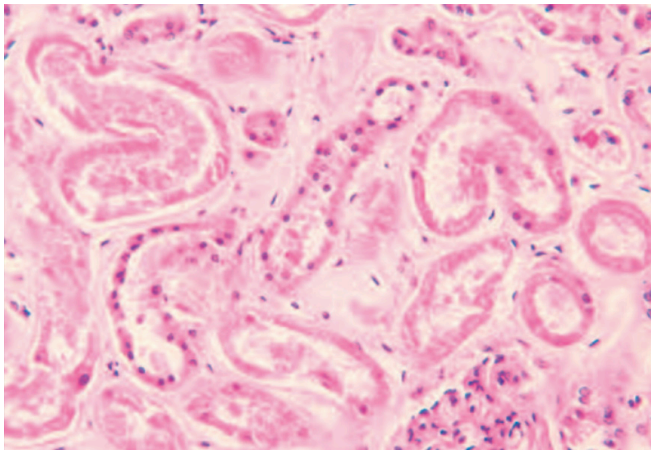
Unfortunately, damaged or lost cells cannot be recovered. If these artefacts are observed in the sample, processes must be immediately improved. Tissue samples should be placed in a fixative solution directly after removal in a volume that is 15 to 20 times that of the tissue, and the solution should be frequently changed.[1] Tissues such as uterus or gastrointestinal tract should be opened so that the fixative can come into direct contact with all surfaces.[1] Larger tissue specimens should be cut into thin slices to ensure total fixative penetration, and cassettes should not be packed too tightly together.[1]

Conversely, it is possible for tissue to be overly fixed. For formalin fixation, too much time spent in the fixative will gradually result in the formation of stable covalent cross-linkages, and a high number of covalent bonds will prevent sufficient antigen retrieval, producing false-negative results.[2] The effects of prolonged fixation and the degree to which immunoreactivity is lost may depend on the antigen of interest.[3]

Official regulatory guidelines for fixation should always be followed. To prevent errors, the time that a specimen is placed into fixative should be recorded, as well as the total time the tissue spent in the fixative.

Figure 1

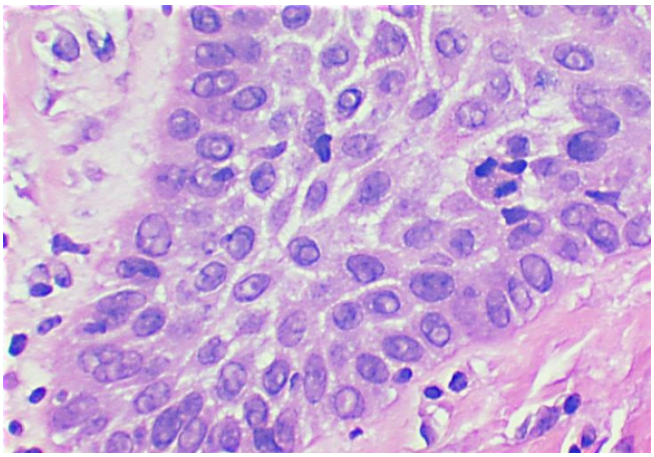
Autolysis on a section of kidney has resulted in a smudgy appearance and the disappearance of nuclei[4]



The effects of mild autolysis can be seen in this section of kidney. From Histologic Preparations: Common Problems and Their Solutions (p. 4), by Richard W. Brown, MD, FCAP, 2009, United States: College of American Pathologists. Copyright 2009

Figure 2

Nuclear bubbling from incomplete fixation can be seen in this skin shave biopsy[5]



Skin Shave Biopsy. From "Nuclear Bubbling- Probable cause(s)/Potential remedies by The College of American Pathologists, 2013, https://webapps.cap.org/apps/docs/proficiency_testing/nuclear_bubbling.pdf Copyright 2013 by College of American Pathologists.

1. Carson, F. L., & Cappellano, C. H. (2009). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP.

2. Thavarajah, R., Mudimbaimannar, V. K., Rao, U. K., Ranganathan, K., & Elizabeth, J. (2012). Chemical and physical basics of routine formaldehyde fixation. Journal of Oral and Maxillofacial Pathology, 16(3), 400. https://doi.org/10.4103/0973-029x.102496

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4. Brown, Richard W. (2009). The effects of mild autolysis can be seen in this section of kidney. [Photograph]. In R. W. Brown, Histologic Preparations: Common Problems and Their Solutions (p. 4). United States: College of American Pathologists, 2009

5. College of American Pathologists. (2013). Skin shave biopsy [Photograph]. Retrieved from https://webapps.cap.org/apps/docs/proficiency_testing/nuclear_bubbling.pdf